STUDIES IN THE PHYSIOLOGY OF THE FUNGI

IV. The Growth of Certain Fungi in Plant Decoctions
Preliminary Account

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While undertaking an extensive study of the nutrition of *Aspergillus niger* in the so-called "synthetic" nutrient solutions it seemed well to determine the growth relations of this fungus and others in various plant decoctions, used either alone or in conjunction with one or more of the more important constituents of the usual synthetic nutrient solution. Numerous studies upon the growth relations of certain fungi with special reference to the sources of carbon and nitrogen have been made by various investigators, likewise considerable work has been done in the direction of determining favorable concentrations of mineral nutrients. Very few data of practical importance have come to the writers' attention bearing upon the nutrient value of the different plant decoctions often employed in culture work. The data here included are of preliminary nature, and merely in respect to a few plant decoctions, but they are sufficiently definite and suggestive to indicate that a complete study of the relations of a considerable number of parasitic and saprophytic fungi to such media would throw valuable light upon many problems which confront the physiologist and pathologist in respect to the artificial cultivation of such fungi. In the study here reported little consideration is given to the relation between vegetation and spore formation, but it is our intention to discuss this point at length in a later report.

Realizing the necessity of adopting some standard in the preparation of plant decoctions it has been the custom in all quantitative work in this laboratory to calculate the amount of the raw vegetable product to be used on the basis of dry weight. The standard employed[1] has been 50 grams dry weight of the product per liter of water, and this has been found to be a satisfactory quantity for most materials. Plant mate-

[1] Duggar, B. M. Fungous diseases of plants. p. 24. 1909.

rials vary so greatly in water content that if any satisfactory standard is adopted it is necessarily on the dry weight basis. Accordingly, for the plant products utilized in preparing the decoctions employed in this work, the quantities required, as calculated from the data in convenient handbooks,[1] are as follows: green (string) beans 391.5 grams; corn meal 58.6 grams; fresh turnips 524.1 grams; sugar beets 370.4 grams; prunes (dried, exclusive of seed) 70.7; and potatoes 236.8.

It is obvious that such plant products will vary more or less in water content depending upon the variety and the season, but this is relatively a minor consideration.

In each case the full weight of material required was cut up into small pieces (not more than 1 cm. in length or diameter), added to 1 liter of water, autoclaved at 15 pounds pressure for 1 hour, filtered hot, and made up to the full quantity, if there had been loss of water. It was determined to use the natural (or native) decoction in each case, and also each one amended as indicated in the series below, these also corresponding to the 7 columns (I–VII) of nutrient media in table 1.

I Natural decoction, full strength.
II Natural decoction, full strength standardized in reaction to + 15 Fuller's scale.
III One-half strength standardized decoction + 13.68% (.4N) cane sugar.
IV One-half strength standardized decoction + 3.42% (.1N) cane sugar.
V One-half strength standardized decoction + 13.68% cane sugar + 1% KNO_3 + .5% KH_2PO_4.
VI One-half strength standardized decoction + 13.68% cane sugar + 1% KNO_3.
VII One-half strength standardized decoction + 13.68% cane sugar + .5% KH_2PO_4.

A part of each decoction was therefore set aside to be used in natural condition while the remainder was titrated,

[1] Jenkins, E. H., and Winton, A. L. A compilation of analyses of American feeding stuffs. U. S. Dept. Agr., Exp. Sta. Bul. 11. 1892.

König, J. Chemische Zusammensetzung der menschlichen Nahrungs- und Genussmittel. Berlin, 1889.

using phenolphthalein as an indicator, and brought to approximately + 15 Fuller's scale,—standard HCl or NaOH being used to obtain the desired reaction.

The reactions of the various natural decoctions on the Fuller scale were as follows: bean + 15, corn meal + 3, turnip + 11.5, sugar beet + 22.6, prune + 14.5, and potato + 11.5. The bean and prune decoctions were left in the "natural" condition, so that the duplicate cultures representing columns I and II for these decoctions were equivalent, and in table 1 the dry weight data are repeated merely for the completion of the table. Immediately after the addition of the required acid or alkali to the other decoctions, a second titration was carried out and a further correction made. Special attention should be drawn to the fact that in I and II full strength decoctions were employed, while in III–VII the decoctions were one-half strength. In later series, not reported upon here, dilution of the decoctions has been avoided, or half strength "control" decoctions also employed.

The cultures were made in duplicate in small Erlenmeyer flasks (125 cc. capacity), each containing 25 cc. of solution. The flasks were sterilized at 15 pounds pressure for 20 minutes.

It seemed desirable to employ fungi with somewhat different habits of growth, including at least one parasitic species, and the following species were chosen, namely, *Macrosporium commune, Aspergillus niger, Glomerella* (*Gloeosporium*) *Gossypii,* and *Penicillium expansum.* Spores were taken from fresh cultures grown 7–10 days on potato agar, except in the case of *Glomerella,* which was grown on bean agar. Under aseptic conditions a strong spore suspension for each organism was made in sterile distilled water, and 4 drops of a suspension were added to each flask in the series for that organism. All cultural operations were executed in a transfer room in which all dust was thoroughly precipitated by steam. No contaminations resulted in any of the 256 cultures made. All the cultures were set up and taken down within one week of each other, while those with any one organism were arranged at the same time and held

at the same temperature for the same interval. The following indicates this:

	Inoculated	Discontinued	No. of days
Macrosporium commune	February 8	February 22	14
Aspergillus niger	February 7	February 21	14
Glomerella Gossypii	February 3	February 20	17
Penicillium expansum	February 7	February 24	17

For all species the temperature variation during the interval of incubation was 20–22° C.

TABLE I

DRY WEIGHTS OF CULTURES ON PLANT DECOCTIONS

Fungus	Weight in grams							Decoction
	I	II	III	IV	V	VI	VII	
	Natural decoction	Standardized decoction	½ strength standardized decoction + 13.68% cane sugar	½ strength standardized decoction + 3.42% cane sugar	½ strength standardized decoction + 13.68% sugar + 1% KNO_3 + .5% KH_2PO_4	½ strength standardized decoction + 13.68% sugar + 1% KNO_3	½ strength standardized decoction + 13.68% sugar + .5% KH_2PO_4	
Macrosporium commune	.119	.119	.562	.295	.....	.....	.....	Bean
Aspergillus niger	.092	.092	.239	.183	.796	.653	.219	
Glomerella Gossypii	.058	.058	.275	.300	.337	.290	.280	
Penicillium expansum	.070	.070	.214	.127	.....	.....	.....	
Macrosporium commune	.068	.018	.059	.060	.....	.....	.....	Corn meal
Aspergillus niger	.055	.086	.116	.085	.492	.171	.144	
Glomerella Gossypii	.069	.025	.094	.052	.139	.101	.096	
Penicillium expansum	.069	.026	.100	.041	.....	.....	.....	
Macrosporium commune	.131	.135	.563	.231	.....	.....	.....	Turnip
Aspergillus niger	.078	.083	.152	.101	.569	.412	.146	
Glomerella Gossypii	.074	.074	.245	.213	.317	.291	.167	
Penicillium expansum	.079	.077	.125	.091	.....	.....	.....	
Macrosporium commune	.370	.257	.493	.275	.....	.....	.....	Sugar beet
Aspergillus niger	.239	.183	.275	.182	.921	.467	.302	
Glomerella Gossypii	.271	.225	.521	.369	.478	.268	.396	
Penicillium expansum	.190	.139	.195	.137	.....	.....	.....	
Macrosporium commune	.111	.111	.628	.285	.....	.....	.....	Prune
Aspergillus niger	.073	.073	.133	.100	.563	.448	.188	
Glomerella Gossypii	.087	.087	.139	.116	.203	.142	.146	
Penicillium expansum	.046	.046	.128	.053	.....	.....	.....	
Macrosporium commune	.088	.088	.661	.308	.....	.....	.....	Potato
Aspergillus niger	.069	.118	.212	.139	.832	.717	.197	
Glomerella Gossypii	.101	.091	.216	.225	.368	.239	.185	
Penicillium expansum	.057	.055	.205	.126	.....	.....	.....	

Growth was satisfactory on all media except the corn meal decoction, yet the amount of growth was considerably less

than anticipated on several of the other media. In further work extensive comparisons will be made between the value of decoctions and some other standard nutrient solutions. On corn meal decoction the growth was particularly unsatisfactory and irregular with *Glomerella* and *Macrosporium*. Moreover, there was gradually deposited in all solutions of this decoction (more in the standardized solutions) a considerable flaky precipitate, and this interfered seriously with correct weight determinations of the mycelium formed, as may be inferred from an examination of table 1.

Filter papers 9 cm. in diameter were dried to constant

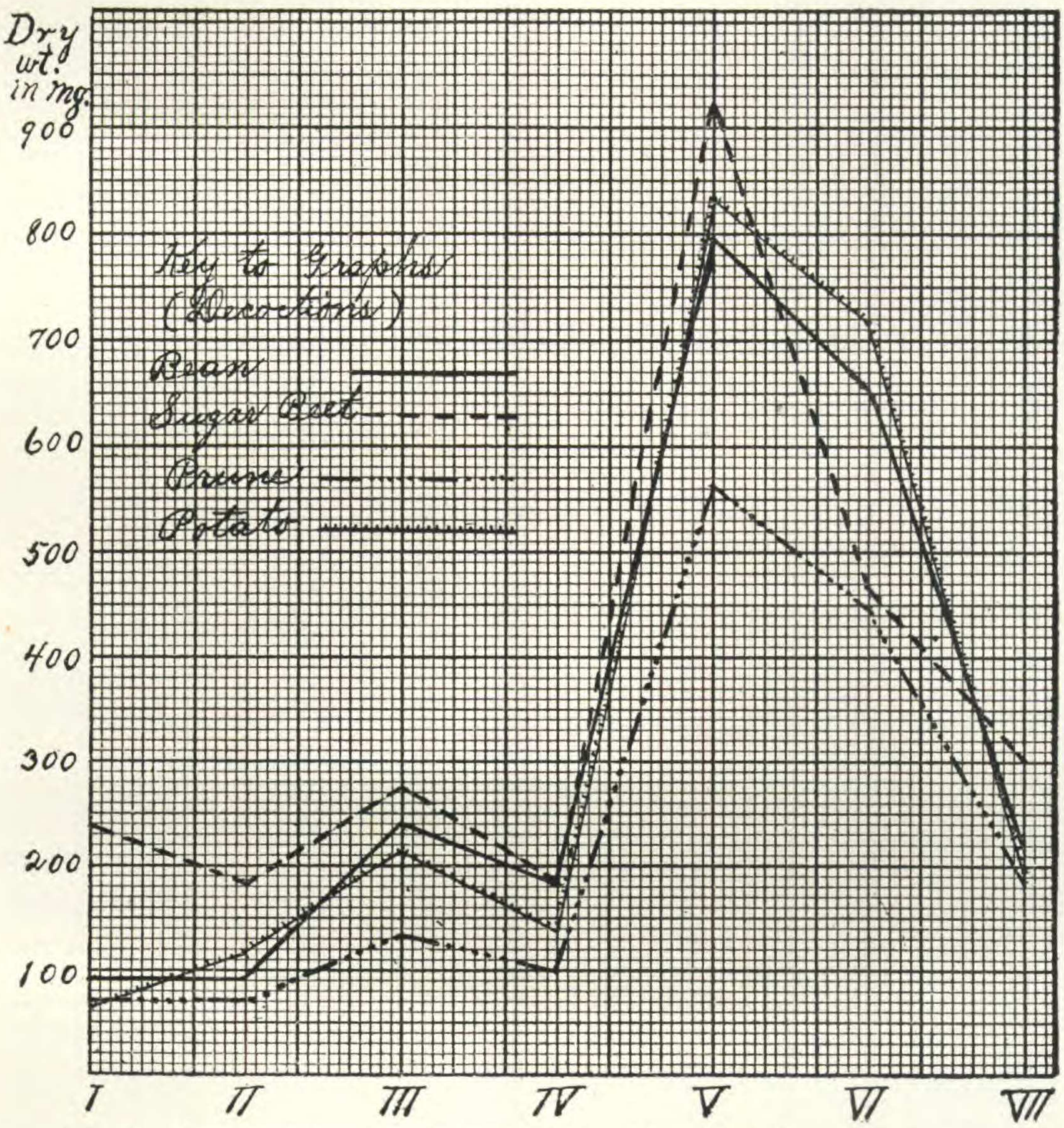

Fig. 1. Graphs showing dry weights of cultures of *Aspergillus niger;* dry weights of felt plotted on ordinates, the solutions (see p. 166 for explanation) on abscissae.

weight at about 105° C., then transferred directly to desiccators with anhydrous and freshly oven-dried $CaCl_2$. After 24 hours they were accurately weighed to the third place and marked with weight and number. As a result of the apparent variations in growth it was determined to make hydrogen ion determinations of the solutions, so that under aseptic conditions the remaining culture fluid was poured off into test-tubes for later use, the mycelial mat being then thrown on to the filter, as also flask washings. The filters were then again dried to constant weight at about 105° C., and placed in desiccators until carefully weighed.

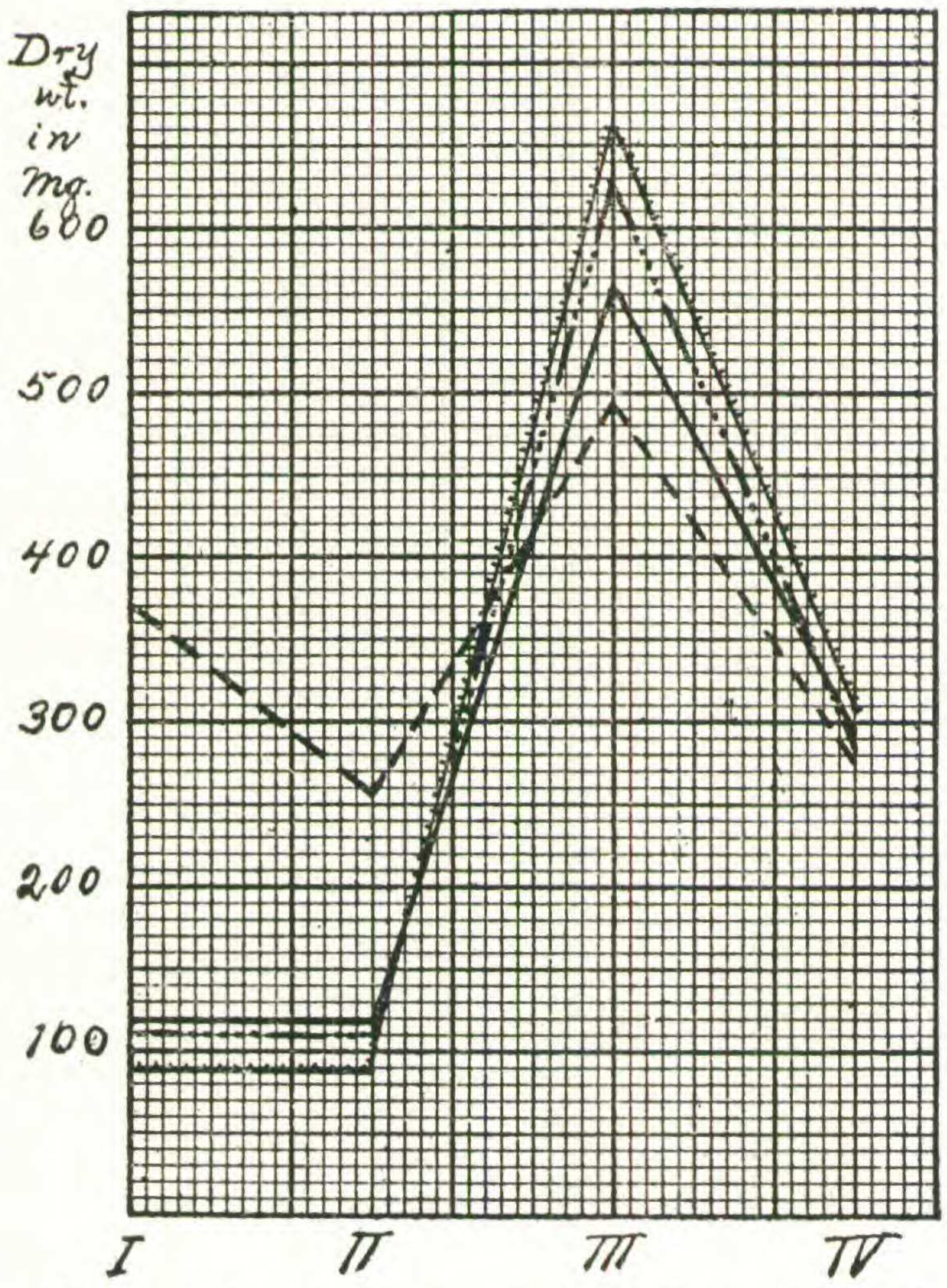

Fig. 2. *Macrosporium commune;* dry weights of cultures in mg. on ordinates, solutions (see p. 166 for explanation) on abscissae. Key to graphs in fig. 1.

In table 1 the average weights of the felts from the duplicate series are given. The results in the duplicate series with all decoctions except corn meal were sufficiently consistent. As mentioned above, in the case of corn meal it was impossible completely to separate the precipitate from the slight mycelial growth, so that the figures in the table are somewhat too large, and, as between duplicate members, there were weight differences not borne out by the record of observations.

The more important data are likewise strikingly shown in

the curves exhibited in figs. 1–4, these representing all four organisms on four of the decoctions, namely, bean, sugar beet, prune, and potato, the data for turnip decoction being omitted merely (a) because it follows very closely in three of the fungi

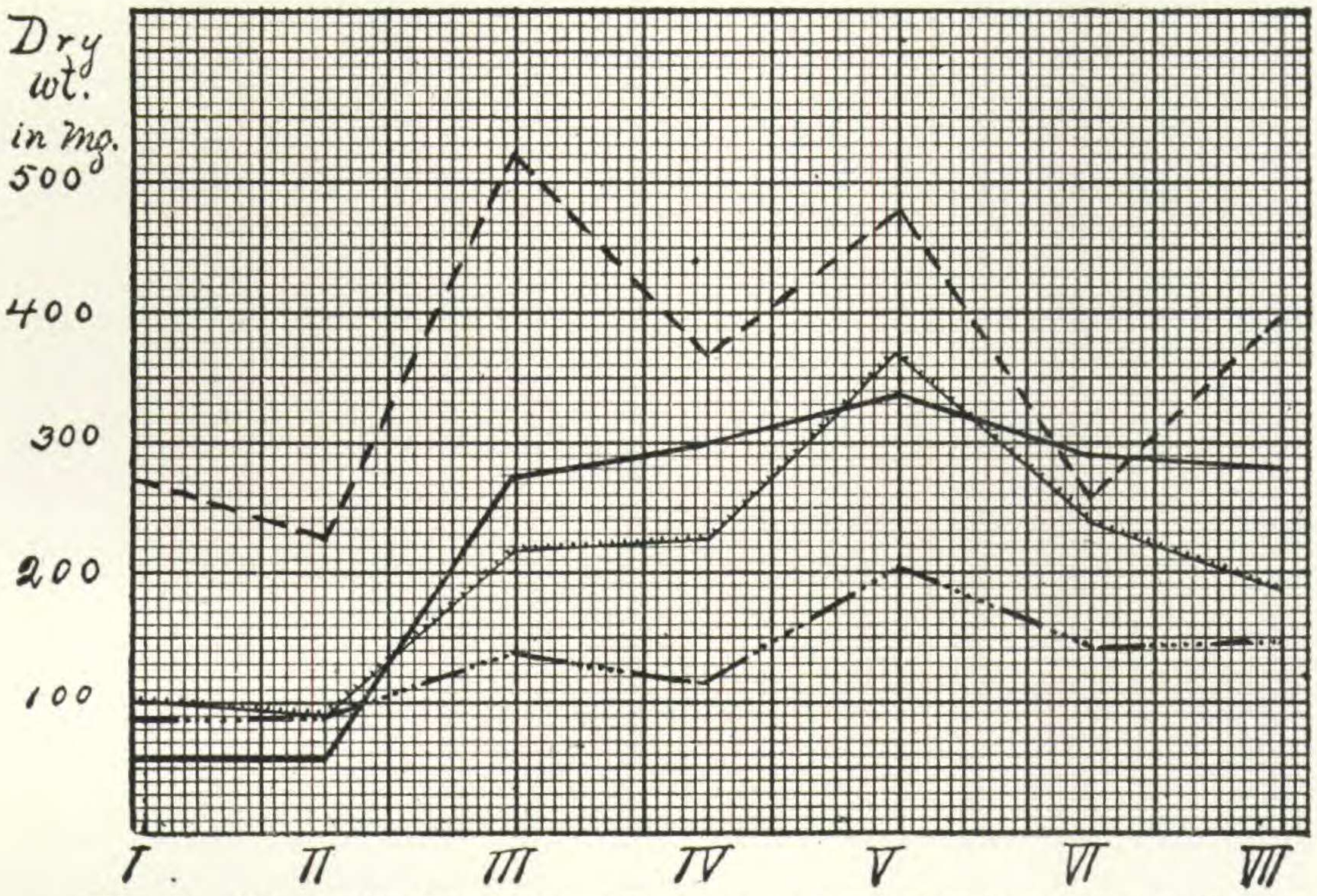

Fig. 3. *Glomerella Gossypii;* dry weights of cultures in mg. on ordinates, solutions (see p. 166 for explanation) on abscissae. Key to graphs in fig. 1.

the curves of the prune decoction, (b) because it would further have complicated the diagrams, and (c) because on the whole it is much less used as a culture medium.

Some of the interesting features of the curves in general are these:

The addition of sugar, nitrate, and phosphate gives in every case except one (*Glomerella* on bean decoction) increase in growth over the addition of sugar alone. In the majority of cases the next highest growth occurs when sugar and nitrate are added. The addition of sugar alone gives a relatively slight increase over the natural decoction, although it is to be remembered that where sugar or other nutrients are added the decoction is diluted one-half. In *Aspergillus* the addition of sugar and phosphate gives a slight increase over the addition of the same concentration of sugar alone. In

the case of *Glomerella* this is variable with the different decoctions. An attempt to standardize the sugar beet decoctions has resulted with every fungus in a slight decrease of growth in comparison with that in the natural decoction. On the whole, the prune decoction has yielded less growth than either of the other three plotted in the curves except in the case of one organism, *Macrosporium.*

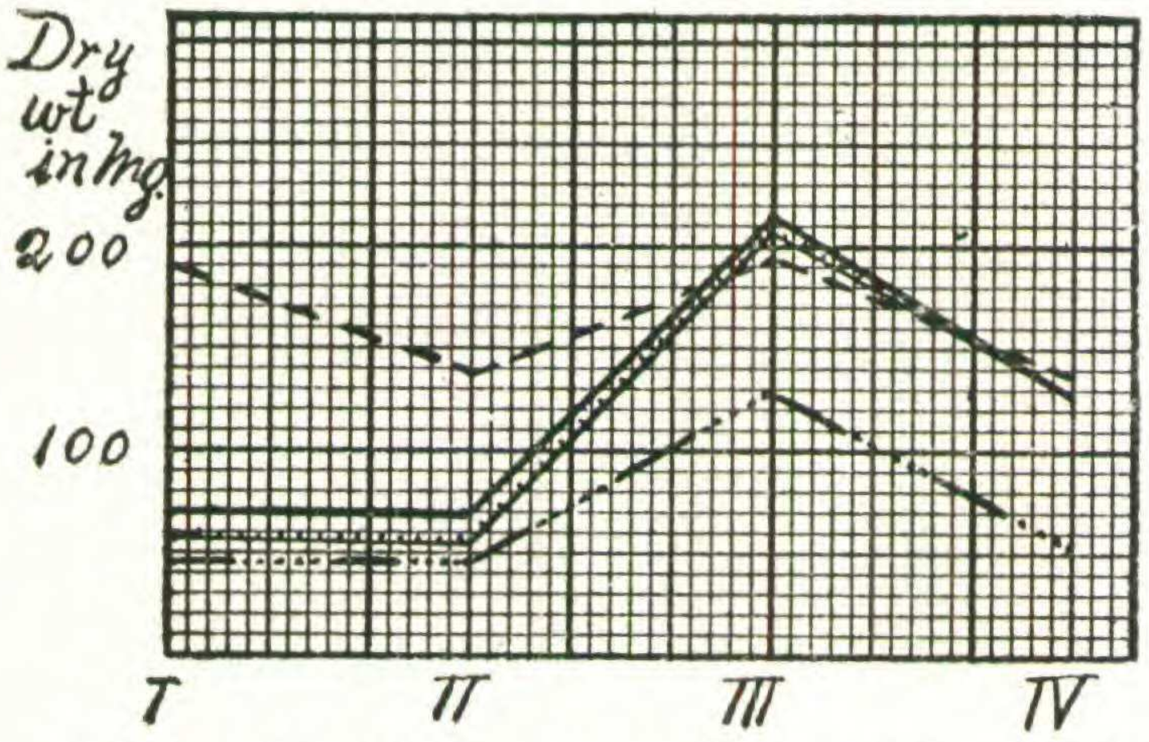

Fig. 4. *Penicillium expansum;* dry weights of cultures in mg. on ordinates, solutions (see p. 166 for explanation) on abscissae. Key to graphs in fig. 1.

Unfortunately, hydrogen ion determinations were not made at the time the cultures were installed, so that in order to obtain results for the original solutions it was necessary to prepare a second lot of the decoctions. These would undoubtedly correspond very closely to those employed for the cultures, and are therefore fairly suitable controls for changes in hydrogen ion concentrations occurring during the growth of the organisms. In this work the colorimetric method was employed, and it is unnecessary here to give the details of the method further than to say that the standard solutions of Sörensen, as modified by Henderson, as well as all available indicators of merit were used.

With reference to the hydrogen ion concentration of the control or original solutions, it is to be noted that little difference was found between the natural decoctions of bean, turnip, prune, and potato, that is, after standardization,—all of these being approximately 10^{-4}. These decoctions, moreover, were only influenced to a slight degree by the addition of sugar or nutrient salts as previously described. After standardization the sugar beet decoction was about 10^{-3} and the corn meal 10^{-2}. It was evident, therefore, that the attempted standardization of corn meal to + 15 Fuller's scale actually

left the solution differing widely from the majority of the decoctions in hydrogen ion concentration.

The changes which were induced in the hydrogen ion concentration in the various solutions as a result of the growth of the different fungi is worthy of mention. In all solutions except the sugar beet and the corn meal decoctions, *Aspergillus* caused, as might be expected, a shift toward the acid side, usually to about 10^{-3}, while *Macrosporium* and *Glomerella* generally induced a pronounced shift in the other direction, these last, however, varying from a scarcely perceptible change in prune decoction to a maximum in the turnip, bean, and potato decoctions, where the test indicated from 10^{-6} to 10^{-8}. In the cultures of *Penicillium* acidity was evidently developed in the bean, turnip, prune, and potato decoction whenever sugar was added, but alkalinity was developed in the natural and standardized decoctions.

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